AN EXPERIMENTAL DEMONSTRATION OF THE REGENERATION OF THE PHARYNX OF ALLOLOBOPHORA FROM ENDODERM.

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SEVERAL recent investigators have shown with more or less probability that the lining of the new pharynx which develops during the regeneration of the head in certain earthworms comes from the endoderm, while the pharynx of the embryo is lined by ectoderm.[1] It seemed that by means of experimental methods this relation might be definitely determined. In the following pages I shall describe some experiments on Allolobophora foetida that demonstrate, I think, that the lining of the new pharynx is in fact derived from the endoderm.

Hescheler showed, as the result of observations made principally on Allolobophora terrestris, that when the five anterior segments are cut off the pharynx is regenerated by a growing forward of the old digestive tract up to the third segment, and that the new buccal cavity occupying the first three segments is formed by an ectodermal invagination. The old pharynx was not completely removed in these operations, since in the normal worm its cavity frequently extends beyond the fifth segment and its thickened muscular dorsal wall always goes back into the sixth, so that Hescheler's results are open to the objection that in his experiments *a part, at least, of the old pharyngeal wall always remained behind* as a possible source for the regeneration of the new pharynx.

Rievel in experimenting on certain Lumbricidae (Allolobophora foetida, Allolobophora terrestris, Lumbricus rubellus) cut off anterior ends consisting of between one-third to two-thirds of the entire body. He arrives at the conclusion that the pharynx is regenerated from the walls of the digestive tract

[1] See Hoffman, *Zeit. f. wiss. Zool.* Bd. lxvi. 1899.

at the point where this was cut, and that no ectodermal invagination whatever occurs, the endodermal diverticulum joining the body wall to form the mouth at the very anterior end of the worm.[1]

Haase showed that in Tubifex, when four to six anterior segments have been removed, the pharynx grows forward out of the walls of the digestive tract and meets an ectodermal invagination of somewhat varying size. This ectodermal pouch, which forms the buccal cavity, is small in all cases, never extending quite as far back even as the region of the cerebral ganglion.

Von Wagner's observations on Lumbriculus show that the point of union of ectoderm and endoderm, originally at the anterior end of the animal, subsequently comes to lie more posteriorly, on account of the forward growth of the "Kopflappen" and accompanying turning in of the ectoderm.

The differences in the accounts cited above show clearly that it is almost impossible to determine with certainty, merely by observation, just how much of the regenerated pharynx ultimately arises from the ectoderm and how much from the endoderm. It is very easy to see where ectoderm and endoderm meet, but the point of fusion is lost soon afterwards, and since the regenerated head continues to increase in size, it is presumably possible that the point of union may come to lie at some distance from its original position. At the time when the pharynx opens to the exterior its walls are not sufficiently developed for one to be able to determine whether the muscles will grow around the endodermal part of the tube; but if in some manner the fusion of the ectoderm with the endoderm could be delayed long enough for the pharyngeal muscles to form around the latter, then the origin of the pharynx might be determined. Hescheler affirms that it is possible to make out the exact limits of the ectoderm by using stains which bring out the cuticle covering this layer. I used the stain which Hescheler mentions as giving the best results, but found that, while my preparations showed in general an agreement

[1] For criticism of Rievel's results, see papers by Morgan (*Roux's Archiv*, Bd. v, 1897) and Hescheler (*Jenaische Zeitschrift*, 1898).

with the figures of Hescheler in regard to the extent of the cuticle on the dorsal wall of the pharynx, they contained also some alternating patches of what seemed to be cuticle and of ciliated areas on the ventral wall and at points in the digestive tract even further back than the regenerated pharynx itself.

For this reason I have attempted to get more certain results by the use of the following experimental methods. Worms, from which the seven anterior segments had been removed, so that no part whatever of the old pharynx was left behind, were allowed to regenerate for a period of between twelve and eighteen days. As a rule the fusion of the ectodermal invagination with the pharynx occurs about fifteen days after the removal of the anterior end of the worm, — although there is considerable individual variation in regard to this point, and also some difference due probably to the temperature, etc. At the end of this time the anterior tip of the new part of the worm was removed in one of two ways: either it was burned off by touching it with a hot needle, or it was cut off with fine scissors. The latter method, though more difficult to carry out successfully, proved to be the better one because the piece cut off could be preserved to show whether the pharynx had joined the ectoderm at the time of the second operation. The worms were once more allowed to regenerate and were finally killed between ten and fifteen days after the second operation. In all cases the worms survived both operations and showed a perfectly normal regeneration, — the only point of difference from worms that had undergone only the first operation being that the new pharynx had time to regenerate before the second ectodermal invagination had fused with its anterior end. The object of the experiment was to determine whether a normal pharynx would develop from the endoderm if the fusion of the ectoderm with it was prevented for a sufficient length of time to allow this development to take place.

It is difficult to determine on the living object whether or not the ectodermal invagination has met the endoderm, and since for my purposes it was best to wait as long as possible before the second operation, it happened in two or three cases,

as sections of the small pieces removed showed, that ectoderm and endoderm had met. In the majority of instances, however, I was fortunate enough to remove the invaginating ectoderm just in time. In cases where this was done with a hot needle there is, of course, nothing to prove that the fusion had not taken place. There is ground for such a belief, however, in the fact that, of a number of worms whose small anterior ends were cut off at the same time after the first operation as when the burning was done, and which were kept under exactly the same conditions, there was not a single one in which the fusion had taken place.

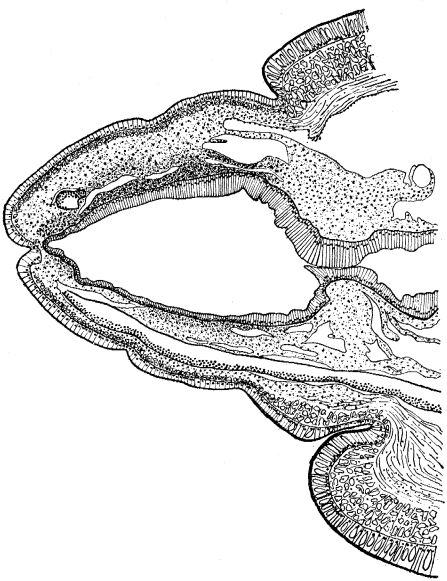

FIG. 1.

The same difficulty presents itself again in determining the time at which the worm is to be killed. I succeeded, however, in getting a number of cases where, though the pharynx and the ectoderm were just on the point of joining, they had not quite done so when the worm was killed. The accompanying figures show two worms in this condition.

Fig. 1 shows a vertical longitudinal section of a worm from which seven segments were removed on January 15. On February 2, that is to say eighteen days later, the tip of the newly regenerated part was cut off. This piece was preserved and sectioned and was found to include the whole of the ectodermal invagination besides the anterior end of the pharyngeal diverticulum which had not yet broken through to the exterior. Fourteen days after this operation, on February 16, the worm was killed.

Fig. 2 represents a vertical longitudinal section of a worm from which the first seven segments were cut off on January

15. The tip of the regenerated part was destroyed with a hot needle on February 2, and the worm was killed on February 17.

Both figures show the diverticulum which has grown out from the walls of the oesophagus about to open to the exterior by fusion with the ectodermal pit; and a comparison with the sections in the same neighborhood shows that these two represent the nearest approach of ectoderm and endoderm to be found in the two specimens. The walls of the pharynx and its musculature, especially on the dorsal side, are well developed. In both worms a nerve cord and a cerebral ganglion have been formed, the latter for the second time. Owing to the slight obliqueness of the section, as shown in Fig. 2, the nerve cord is cut for only a part of its length. The muscles of the body wall have begun to differentiate and there are clear indications of metamerism. All of the worms used in this set of experiments, as well as all those in a later set made to test these results, present a similar condition of things.

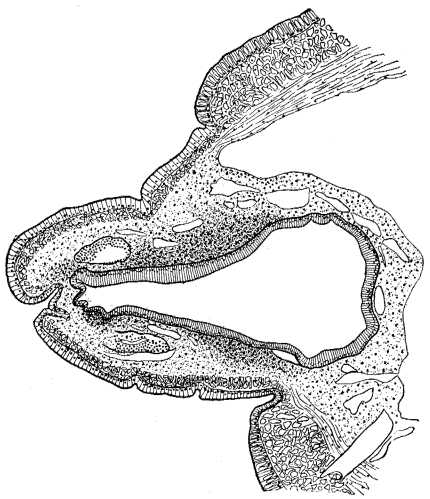

FIG. 2.

From these results we must conclude that the lining of the pharynx is regenerated from the endoderm, while the new ectoderm turns in for a very short distance to meet the pharynx and form the mouth.

The objection may be raised that the possibility of a later pushing in of the ectoderm to form the ultimate lining of the pharynx is in no way removed. But there is no evidence for such an occurrence and, even if it did take place, the fact remains that the musculature of the pharynx develops around an endodermal tube, as my experiments have shown, while in

the embryo the lining of this same region is derived from the ectoderm.

The preceding work was done under the direction of Prof. T. H. Morgan, to whom I wish to express my indebtedness.

BRYN MAWR, May 26, 1900.